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(54) Title: PRION PROCESSING

(57) Abstract: A method for processing prions is disclosed, comprising contacting at least one porous medium with a pathogenic prion-containing fluid such that pathogenic prions are retained and/or captured by the porous medium; contacting the porous medium with another fluid such that pathogenic prions are released from the porous medium to provide a first released pathogenic prion-containing fluid; placing a fluid containing the released pathogenic prions in a first spin device including a separation medium; and, centrifuging the spin device to provide a first concentrated pathogenic prion-containing retentate.

PRION PROCESSING

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 60/818,958, filed July 7, 2006, which is incorporated by reference.

BACKGROUND OF THE INVENTION

[0002] Conformational diseases are a group of disorders apparently unrelated to each other, but sharing a similarity in clinical presentations that reflect their shared molecular mechanism of initiation and self-association, with consequent tissue deposition and damage. These varied diseases each arise from an aberrant conformational transition in an underlying protein, characteristically leading to protein aggregation and tissue deposition. Two examples of conformational diseases are Alzheimer dementia, and Transmissible Spongiform Encephalopathies (TSE), also known as prion disease or "mad cow disease."

[0003] Prions are infectious pathogens causing TSE in humans and animals. It is believed prions are devoid of informational nucleic acids, and that they consist of an infectious protein, termed PrP^{Sc} (generated from the normal cellular form of the protein, PrP^{C}), capable of converting the normal form of the protein into an infectious protein. The disease related form, PrP^{Sc} (sometimes referred to as PrP^{Res}) can typically be distinguished from the normal cellular form, PrP^{C}, by, for example, the disease form's insolubility and partial resistance to proteases. The disease related and normal cellular forms differ in their tertiary structure, as PrP^{C} has a predominantly α-helical conformation, and PrP^{Sc} (or PrP^{Res}) has a predominantly β-sheet conformation.

[0004] Prion-caused spongiform encephalopathies in humans include, for example, Creutzfeldt-Jacob disease (CJD, and variants thereof), Gerstmann-Straussler-Scheinker Syndrome (GSS), Kuru, Alpers Syndrome, and fatal familial insomnia (FFI)). Spongiform encephalopathies in animals include, for example, scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, transmissible mink encephalopathy (TME) in mink, and chronic wasting diseases (CWDs) in mules, deer, and ungulates.

[0005] It is believed prions can possibly be transmitted by prion-contaminated fluids, such as blood products, and pharmaceuticals (e.g., prion-contaminated hormones derived or extracted from infected hosts). Accordingly, some material suspected of containing prions

has been destroyed. Additionally, in the United States, the American Red Cross's donor eligibility rules exclude accepting donations from those who have spent a considerable time in various countries where variant Creutzfeldt-Jacob disease (vCJD) or mad cow disease has been found.

[0006] While there is great interest in the detection and/or analysis of TSE in humans and other animals, detection, e.g., by assaying for a marker such as PrP^{Sc}, has been difficult, since, for example, levels of the marker are typically too low for detection with conventional methods until the disease has reached a late stage.

[0007] Accordingly, there is a need for methods, systems, and devices for processing possibly infectious prion-contaminated fluid to remove prions from the fluid and/or concentrate the pathogenic prions in a fluid, e.g., for detection and/or analysis.

[0008] The present invention provides for ameliorating at least some of the disadvantages of the prior art. These and other advantages of the present invention will be apparent from the description as set forth below.

BRIEF SUMMARY OF THE INVENTION

[0009] An embodiment of the invention provides a method for concentrating pathogenic prions comprising contacting at least one porous medium with a pathogenic prion-containing fluid such that pathogenic prions are retained and/or captured by the porous medium; contacting the porous medium with another fluid such that pathogenic prions are released from the porous medium to provide a released pathogenic prion-containing fluid; placing a fluid containing the released pathogenic prions in a spin device including a separation medium; and, centrifuging the spin device to provide a concentrated pathogenic prion-containing retentate.

[0010] In another embodiment, a system for concentrating pathogenic prions is provided comprising a prion capture/release device comprising a housing having (a) at least one inlet and at least one outlet and defining a fluid flow path between the inlet and the outlet; and at least one porous medium across the fluid flow path, the porous medium comprising a pathogenic prions retaining and/or capturing medium, or (b) having a plurality of wells wherein at least two wells each have at least one porous medium sealed therein, the porous medium comprising a pathogenic prions retaining and/or capturing medium; and, a spin device including a separation medium, wherein the spin device is adapted to be centrifuged to provide a concentrated pathogenic prion-containing retentate.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWING(S)

[0011] Figure 1 shows assembled (Figure 1A), cross-sectional (Figure 1B), and exploded (Figure 1C), views of an embodiment of a pathogenic prion capture device in accordance with the present invention.

[0012] Figure 2 shows exploded (Figure 2A), and cross-sectional (Figure 2B), views of another embodiment of a pathogenic prion capture device in accordance with the present invention.

[0013] Figure 3 is an embodiment of a system for capturing and releasing prions in accordance with the present invention.

DETAILED DESCRIPTION OF THE INVENTION

[0014] In accordance with an embodiment of the present invention, a method for concentrating pathogenic prions comprises contacting at least one porous medium with a pathogenic prion-containing fluid such that pathogenic prions are retained and/or captured by the porous medium; contacting the porous medium with another fluid such that pathogenic prions are released from the porous medium to provide a released pathogenic prion-containing fluid; placing a fluid containing the released pathogenic prions in a spin device including a separation medium; and, centrifuging the spin device to provide a concentrated pathogenic prion-containing retentate.

[0015] In another embodiment, a system for concentrating pathogenic prions is provided comprising a prion capture/release device comprising a housing having at least one inlet and at least one outlet and defining a fluid flow path between the inlet and the outlet; and at least one porous medium across the fluid flow path, the porous medium comprising a pathogenic prion retaining and/or capturing medium; and, a spin device including a separation medium, wherein the spin device is adapted to be centrifuged to provide a concentrated pathogenic prion-containing retentate.

[0016] In yet another embodiment, a system for concentrating pathogenic prions is provided comprising a prion capture/release device comprising a housing having a plurality of wells wherein at least two wells each having at least one porous medium sealed therein, the porous medium comprising a pathogenic prion retaining and/or capturing medium; and, a

spin device including a separation medium, wherein the spin device is adapted to be centrifuged to provide a concentrated pathogenic prion-containing retentate.

[0017] Typically, the eluted prions, more typically, the concentrated prions, are further treated, e.g., passed through an electrophoresis gel. The prions can be analyzed, e.g., via Western blotting.

[0018] In a preferred embodiment, the pathogenic prions can be concentrated without precipitation chemistry treatment. Alternatively, or additionally, pathogenic prions can be concentrated without pK treatment and/or the use of specific ligands to eliminate/minimize the presence of non-pathogenic prions.

[0019] In some embodiments, the pathogenic prions are concentrated as part of an automated protocol.

[0020] Embodiments of the invention are suitable for use in, for example, laboratories and/or hospitals, and can be used for detection, analysis, diagnosis and/or treatment. In addition to use with humans, a variety of veterinary applications are also encompassed by embodiments of the invention.

[0021] Moreover, embodiments of the invention are suitable for concentrating pathogenic conformers other than pathogenic prions, as noted in more detail below.

[0022] Yet another embodiment of the invention comprises devices, methods, and systems for removing pathogenic conformers, preferably, pathogenic prions, from a pathogenic conformer-containing fluid. For example, one embodiment of the invention provides a prion capture device. In a more preferred embodiment, the pathogenic conformers are removed from the pathogenic conformer-containing fluid by capturing/retaining them by a porous medium, and the pathogenic conformers are subsequently released from the porous medium to provide a released pathogenic conformer-containing fluid, e.g., comprising enriched prions. Thus, illustratively, another embodiment of the invention provides a prion capture and release device. Even more preferably, the released pathogenic conformer-containing fluid is processed to concentrate the pathogenic conformers in a fluid.

[0023] In accordance with the invention, the pathogenic prion-containing fluid is placed in contact with one or more porous media such that pathogenic prions are retained and/or captured by the porous medium or media. In some embodiments, one or more reagents can be added to the pathogenic prion-containing fluid before placing a pathogenic prion-containing fluid in contact with the porous medium or media. For example, the pathogenic prion-containing fluid can be mixed with one or more of the following, e.g.,

individually or in any combination: one or more salts (e.g., 1 M NaCl), detergents (e.g., a non-ionic detergent such as, for example, Tween-20) and chaotropic agents (such as, for example, 2M urea), and the modified pathogenic prion-containing fluid is subsequently placed in contact with the porous medium/media such that the pathogenic prions are retained and/or captured.

[0024] For example, the porous medium or media can be placed in a housing to provide a capture device or a capture/release device, e.g., a prion capture device or a prion capture/release device comprising a housing having at least one inlet and at least one outlet and defining a fluid flow path between the inlet and the outlet, wherein at least one porous medium is interposed between the inlet and the outlet and across the fluid flow path, and a pressure differential is created (e.g., by gravity, negative pressure, or positive pressure) such that substantially all of the fluid passes through the porous medium and the pathogenic prions are retained and/or captured. In accordance with another illustrative embodiment, e.g., wherein the prion capture device or prion capture/release device comprises a housing having at least one inlet and at least first and second outlets and defining a first fluid flow path between the inlet and the first outlet, and defining a second fluid flow path between the inlet and the second outlet, wherein at least one porous medium is interposed between the inlet and the second outlet and across the second fluid flow path, and a pressure differential is created such that a portion of the fluid passes tangentially to the surface of the porous medium and another portion passes through the porous medium, and the pathogenic prions are retained and/or captured. In yet another embodiment, the prion capture device or prion capture/release device comprises a multiple well device wherein at least one porous medium is disposed in each of a plurality of wells, and a pressure differential is created such that substantially all of the fluid passes through the porous medium in an individual well and the pathogenic prions are retained and/or captured.

[0025] Typically, the pathogenic prion-containing fluid placed in contact with at least one porous medium comprises a fluid containing pathogenic prions and non-pathogenic prions, and the method includes contacting at least one porous medium with the pathogenic and non-pathogenic prion-containing fluid such that pathogenic prions are retained and/or captured by the porous medium and non-pathogenic prions are essentially not retained and/or captured by the medium.

[0026] Without being bound to any particular mechanism, it is believed preferred embodiments of the prion capture device and the prion capture/release device according to

the invention retain both pathogenic prions that are associated with cells (e.g., leukocyte-associated prions), and pathogenic prions that are not associated with cells.

[0027] For example, at least one porous medium utilized (e.g., in a prion capture device or a prion capture/release device) in a preferred embodiment of the invention comprises a positively charged medium, more preferably, a positively charged leukocyte depletion medium, which depletes leukocytes from the fluid passing through the medium. Since some of the prions bind to leukocytes (e.g., binding to glycosyl phosphatidylinositol (GPI) anchors), depleting the fluid of leukocytes also depletes the fluid of the leukocyte-associated (cell-associated) prions. Additionally, or alternatively, at least one porous medium utilized in an embodiment of the invention comprises a charged medium (as described in more detail below), and media with different charges and/or different combinations of charges, e.g., at least one positively charged medium and at least one negatively charged medium, can be used in accordance with embodiments of the invention. It is believed some prions are removed from the fluid passing through the medium or media via one or more of the following, e.g., individually, or in any combination of two or more: ionic interaction, hydrophobic interaction, protein-protein interactions and hydrogen bonding. In some embodiments, at least one charged medium comprises a leukocyte depletion medium. Alternatively, or additionally, in some embodiments, at least one medium, that can comprise a leukocyte depletion medium and/or a charged medium, can comprise a hydrophobic domain, and, without being bound to any particular theory, it is believed some prions are removed from the fluid passing through the medium or media via hydrophobic interaction with a hydrophobic domain present in the medium/media. In some embodiments, pathogenic prion-containing aggregates are formed, and the aggregates are depleted from the fluid passing through the medium or media via sieving and/or depth filtration.

[0028] In accordance with an embodiment of the invention including a prion capture/release device, the pathogenic prions (and/or other pathogenic conformers such as amyloid proteins) retained/captured by the porous medium or porous media during the processing of the pathogenic conformer-containing fluid are subsequently released from the medium or media and recovered, for example, by washing (e.g., eluting) the prions (and/or amyloid proteins) from the medium or media. A variety of processes and fluids for releasing retained pathogenic conformers are suitable for carrying out the invention. Thus, for example, prions can be released by diffusion from the porous medium into the releasing fluid (e.g., an elution fluid). Desirably, fluid flow is applied to the prions retained on the porous

medium to facilitate their dissociation from the porous medium by creating shear forces on the prions relative to the porous medium. Thus, for example, the prions of interest can be released by passing the elution fluid transversely across the upstream surface of the medium, passing the elution fluid from the upstream surface of the medium to the downstream surface, or backflushing the medium by passing the fluid from the downstream surface to the upstream surface.

[0029] One or more fluids containing released prions are preferably subsequently processed to concentrate the prions by centrifugation in a spin device including a separation medium to provide a first concentrated prion-containing retentate. If desired, the first concentrated prion-containing retentate can be further processed, e.g., to provide a fluid that is centrifuged in another spin device including a separation medium to provide at least a second (or additional) concentrated prion-containing retentate.

[0030] Optionally, one or more fluids containing released prions can be processed before placing a prion-containing fluid in a spin device including a separation medium, e.g., a prion-containing fluid can be centrifuged so that the prions are concentrated in a sediment fluid or a pellet, and, after separating the sediment fluid or pellet from the supernatant, the prions can be resuspended in a lower volume of fluid. Prions can be concentrated in a sediment fluid or pellet and resuspended in a lower volume of fluid more than once before placing a prion-containing fluid in a spin device including a separation medium.

[0031] A variety of spin devices including separation media are suitable for use in embodiments of the invention. Suitable commercially available spin devices include, for example, centrifugal devices available from Pall Corporation (East Hills, NY) as NANOSEP® Centrifugal Devices and MICROSEP™ Centrifugal Devices. Alternatively, suitable commercially available spin devices include, for example, multiple well centrifugal devices or filter plates, e.g., from Pall Corporation under the tradenames ACROWELL™ and ACROPREP™ and/or described in, for example, International Publication No. WO 2002/096563.

[0032] A variety of separation media are suitable for use in spin devices in accordance with embodiments of the invention. Preferred media are membranes, more preferably, low protein binding membranes. In some embodiments, the membranes are ultrafiltration membranes. For example, suitable membranes are 5,000 molecular weight cut off (mwco) or greater, preferably, 30,000 mwco or greater, e.g., 50,000 mwco, 75,000 mwco or greater, or 100,000 mwco, or greater. Typically, the membranes are about 300,000 mwco or less, e.g.,

in the range of from about 10,000 mwco to about 250,000 mwco, or in the range of from about 30,000 mwco to about 150,000 mwco.

[0033] As used herein, the term pathogenic conformer when referring to prions refers to the constrictive form of the prion (e.g., PrP^{Sc}), and can include one or more particular PrP^{Sc} isoforms, and protease-sensitive and protease-resistant conformers (e.g., $sPrP^{Sc}$ and $rPrP^{Sc}$). Although the PrP^{Sc} form of a PrP protein is infectious and pathogenic, the disease conformation of other proteins can be non-infectious although it is pathogenic. Thus, the term pathogenic can mean the protein actually causes the disease or it can mean the protein is associated with the disease and therefore is present when the disease is present.

[0034] As used herein, the term non-pathogenic conformer when referring to prions refers to the relaxed form of the prion (e.g., PrP^{C}).

[0035] In the context of amyloid proteins, with respect to Alzheimer's disease, the term pathogenic conformer refers to the pathogenic form of β-amyloid protein, and non-pathogenic conformer refers to the non-pathogenic form of β-amyloid protein. Embodiments of the invention also encompass concentrating pathogenic conformers related to other amyloid proteins, e.g., implicated in other forms of amyloidosis such as systemic amyloidosis (e.g., primary systemic amyloidosis and/or secondary systemic amyloidosis), familial amyloid polyneuropathy (e.g., familial amyloid polyneuropathy I and/or familial amyloid polyneuropathy III), diabetes, and various cancers (e.g., medullary thyroid carcinoma).

[0036] The possibly pathogenic prion-containing fluid utilized in accordance with embodiments of the invention can have any suitable volume. For example, a greater volume of fluid may be used when processing a fluid for medical use, e.g., when filtering a biological fluid such as a blood product to provide a pathogenic prion-depleted fluid for eventual use in a transfusion, than when processing a fluid for a laboratory or diagnostic application. Typically, with respect to contacting at least one porous medium with a pathogenic prion-containing fluid to deplete the fluid of pathogenic prions (e.g., passing the fluid through a prion capture device or through a prion capture/release device) at least about 5 ml is used, and in some embodiments, at least about 50 ml is used.

[0037] A variety of possibly pathogenic prion-containing fluids can be utilized in accordance with the embodiments of the invention, e.g., biological fluids, fluids containing and/or derived from a tissue and/or fluids suitable for use in the pharmaceutical industry, e.g., carrier fluids such as saline. Alternatively, or additionally, the fluid could be a fluid for human or animal consumption, e.g., a food product or drink (for example, but not limited to,

milk or milk products), wherein it is desired to establish the presence or absence of pathogenic conformers therein. A variety of tissues can be utilized in accordance with the invention, e.g., brain tissue, spinal cord, tonsillar tissue or appendix tissue. In one preferred embodiment wherein the fluid is derived from a tissue, the tissue is brain tissue, e.g., the brain tissue, or a homogenate or a fraction derived therefrom, can be processed to provide a fluid to be placed in contact with a pathogenic conformer-retaining porous medium in accordance with the invention. In some preferred embodiments, the fluid comprises a biological fluid, such as, for example, blood or blood products. The tissue or biological fluid can be from a human, or from an animal, e.g., rodents and ungulates. An illustrative animal includes, but is not limited to a dog, cat, sheep, goat, cow, mink, mule, deer, horse, hamster, mouse, or rat.

[0038] A biological fluid includes any treated or untreated fluid associated with living organisms, particularly blood, including whole blood, warm or cold blood, and stored or fresh blood; treated blood, such as blood diluted with at least one physiological solution, including but not limited to saline, nutrient, and/or anticoagulant solutions; blood components, such as platelet concentrate (PC), platelet-rich plasma (PRP), platelet-poor plasma (PPP), platelet-free plasma, plasma, fresh frozen plasma (FFP), components obtained from plasma, packed red cells (PRC), transition zone material or buffy coat (BC); blood products derived from blood or a blood component or derived from bone marrow; stem cells; red cells separated from plasma and resuspended in physiological fluid or a cryoprotective fluid; and platelets separated from plasma and resuspended in physiological fluid or a cryoprotective fluid. The biological fluid may have been treated to remove some of the leukocytes before being processed according to the invention. As used herein, blood product or biological fluid refers to the components described above, and to similar blood products or biological fluids obtained by other means and with similar properties. Multiple units of some blood components, particularly platelets and buffy coat, may be pooled or combined, typically by combining four or more units.

[0039] If desired, a pathogenic conformer-containing fluid and/or tissue can be treated to, for example, initially concentrate the pathogenic conformer in the fluid and/or tissue derived fluid before placing the pathogenic conformer in contact with the pathogenic conformer-retaining porous medium. For example, since PrP^{Sc} may be preferentially located in a region of the plasma membrane which is resistant to mild detergent (e.g., cold Triton X-100) treatment, this region or domain of the membrane, sometimes referred to as the

lipid-raft, detergent-resistant membrane (DRM) or caveolae-like domain (CLD), lipid raft isolation can be utilized to concentrate or enrich the level of PrP^{Sc} present before placing it in contact with the porous medium or media. After the PrP^{Sc} is released from the medium or media, it can be further concentrated in accordance with the invention.

[0040] If desired, before releasing the captured/retained pathogenic prions from the porous medium or media, the media can be contacted with a rinsing or washing fluid to remove contaminants. Thus, when the pathogenic prions are subsquently released, contamination is reduced.

[0041] A variety of releasing fluids, e.g., elution fluids, can be used in carrying out the invention. Illustratively, an elution fluid with a suitable pH (e.g., under denaturing conditions) and/or ionic strength can be used. Alternatively, or additionally, the elution fluid can vary in other respects such as osmolality, electrolyte concentration, and other parameters. Moreover, the elution fluid can include additional elements as well, such as sugars (including, for example, monosaccharides, disaccharides, and sugar alcohols), buffers (e.g., phosphate buffers, citrate buffers, etc.), enzymes (e.g., proteases), polymers, detergents (e.g., ionic and/or non-ionic detergents) and/or other additives.

[0042] Typically, the elution fluid, hereinafter referred to as a pathogenic conformer releasing solution, includes at least one agent from the group consisting of a chaotropic agent, a non-ionic agent, and an ionic agent. The fluid can include any combination of two or more agents, e.g., a chaotropic agent and a non-ionic agent and/or, for example, two or more chaotropic agents. In an embodiment, the pathogenic conformer releasing solution includes a chaotropic agent, a non-ionic agent, and an ionic agent.

[0043] Illustrative chaotropic agents include, but are not limited to, ethylene glycol and polyethylene glycol. Illustrative non-ionic and ionic agents include detergents and surfactants. For example, illustrative non-ionic detergents and surfactants include, but are not limited to, Nonidet P-10 (NP 10), Nonidet P-40 (NP 40), MEGA-8, MEGA-9, MEGA-10, Igepal CA-630 (Rhone-Poulenc, Inc.), TWEEN 20, TWEEN 40, TWEEN 60, TWEEN 80, and Triton® X-100 (SIGMA® Chemical Company). Illustrative ionic detergents include, but are not limited to, sarkosyl (sodium lauryl sarcosinate), digitonin, TOPPS (*tert*-octyl phenyl propanesulfonic acid), CTAB (cetyltrimethylammonium bromide), TDTAB (tetradecyltrimethylammonium bromide), and SDS (sodium dodecyl sulfate).

[0044] Alternatively, or additionally, in some embodiments, the pathogenic conformer releasing solution includes at least one zwitterionic detergent such as, but not limited to,

BigCHAP, CHAPS, ZWITTERGENT 3-08, ZWITTERGENT 3-10, ZWITTERGENT 3-12, ZWITTERGENT 3-14, and ZWITTERGENT 3-16.

[0045] In some embodiments, the pathogenic conformer releasing solution comprises a salt solution, e.g., including, but not limited to, sodium chloride and/or potassium chloride, at any suitable concentration. For example, the releasing solution can comprise in the range of from about 1% to about 20% salt solution, in some embodiments, in the range of from about 3% to about 12% salt solution. Illustratively, the pathogenic conformer releasing solution can comprise in the range of from about 3% to 12% sodium chloride or potassium chloride solution. Typically, an embodiment of the pathogenic conformer releasing solution comprises a salt solution including an ionic detergent.

[0046] Preferably, as noted above, the pathogenic conformers are released by backflushing from the porous medium or media. Backflushing is preferred, as it is believed the greater concentration of pathogenic conformers is generally on the upstream surface of the porous medium. However, in accordance with embodiments of the invention, the medium can be flushed in either direction (forward flushing being passing the elution fluid through the porous medium in a direction from the upstream side towards the downstream side, backflushing being the reverse). In some embodiments, recovery efficiency is further enhanced by forward-flushing the filter medium in combination with backflushing. Thus, for example, after backflushing the medium with a volume of elution fluid, the medium can then be forward-flushed with a second volume of elution fluid.

[0047] The flushing can be accomplished at any suitable fluid flow rate, e.g., about 0.1-15 L/min/m^2, although flow rates significantly more or less than this range can be used. For example, backflushing can be accomplished at a fluid flow rate of about 0.5-10 L/min/m^2, such as about 1-8 L/min/m^2; more preferably the flow rate is about 1.5-7 L/min/m^2, such as about 2-6 L/min/m^2 or even about 2.5-5 L/min/m^2 (e.g., about 3-4 L ml/min/m^2). The most preferable flow rate depends upon the viscosity and temperature of the elution fluid, and the nature of the porous medium. Thus, in some applications, such as when more gentle treatment is desired, backflushing can be accomplished at a flow rate about 1-100 ml/min/m^2, (e.g., about 15-85 ml/min/m^2); more preferably the flow rate is about 30-70 ml/min/m^2 or even about 40-60 ml/min/m^2 (e.g., about 50 ml/min/m^2).

[0048] Additionally, in some embodiments, the flushing (forwardflushing and/or backflushing) can include pulsing the flow of the flushing fluid.

[0049] In some embodiments wherein the capture/release device includes one or more supports downstream of the medium/media (i.e., the upstream surface of the medium/media is the surface first contacted by the prion-containing fluid) and backflushing is utilized, it may be advantageous to permit the medium/media to deflect away from the downstream supports. Alternatively, or additionally, in some embodiments including forward flushing, the capture/release device includes one or more supports upstream of the medium/media to permit the medium/media to deflect away from the upstream supports. In some embodiments, during deflection, the medium can be distorted and/or stretched, as long as this does not significantly impinge on the housing itself and reduce flow. Without being bound to any particular theory, it is believed this further reduces the prion's attachment to the medium/media during reverse or forward flow, thus enhancing release.

[0050] A variety of systems and arrangements can be utilized to place the pathogenic conformer releasing solution in contact with the porous medium, e.g., to elute the pathogenic prions from the porous medium. Typically, the system and/or arrangement allows creating a pressure differential between the upstream and downstream surfaces of the medium (which can include flowing pathogenic conformer releasing solution tangentially to a surface of the medium). The pressure differential can be created using at least one of gravity, one or more pumps, one or more expressors (including, but not limited to, the expressor disclosed in US Patent No. 5,690,815), and one or more syringes. One or more flow control devices such as valves can be disposed upstream and/or downstream of the porous medium for use in directing flow in a desired direction, e.g., toward or away from the porous medium. Alternatively, or additionally, one or more fluid delivery devices such as pipettes can be disposed upstream and/or downstream of the porous medium for use in directing flow in a desired direction, e.g., toward or away from the porous medium.

[0051] In an illustrative embodiment of a system, wherein a prion capture/release device is provided including a housing having an inlet and an outlet and defining a fluid flow path between the inlet and the outlet, and a prion retaining and/or capturing porous medium is disposed across the fluid flow path, syringes and valves are placed in communication with the inlet and outlet of the device, so that, when desired, pathogenic conformer recovery solution is backflushed via the outlet through the medium, and the eluted pathogenic conformers are recovered via the inlet.

[0052] In another illustrative embodiment of a system, wherein a prion capture/release device is provided including a housing having a plurality of wells, and a prion retaining

and/or capturing porous medium is disposed in each of at least two cells, fluid delivery devices (e.g., pipettes) are placed in communication with one or more wells, so that, when desired, pathogenic conformer recovery solution is added the medium/media, and the eluted pathogenic conformers are recovered from the appropriate well(s).

[0053] One or more porous media, typically disposed in a housing to provide a prion capture device or a prion capture/release device, can be used in accordance with the invention. In some embodiments, a filter, comprising a least one porous filter element comprising at least one porous medium, is disposed in a housing to provide a prion capture device or a prion capture/release device. In those embodiments wherein the prion capture device or prion capture/release device comprises a multiple well device, one or more porous media can be disposed in each of two or more wells.

[0054] In some embodiments, the capture device or capture/release device includes two or more porous media, two or more layers of porous media, or one or more homogenous or non-homogenous porous media. In those embodiments including two or more layers of porous media, two or more layers can have different characteristics, or the same or essentially the same characteristics. In those embodiments having non-homogenous porous media, portions or sections of a given medium may differ from other portions or sections of the medium with respect to, for example, one or more of average fiber diameter, voids volume, and/or pore structure (e.g., pore rating). Without being bound to any particular theory, it is believed that the use of more than 1 layer or medium allows one or more of the following: better dispersion, more effective use of the effective chromatography area (ECA) of the medium/media, and less channeling of the liquid through the medium/media.

[0055] At least one porous medium and/or at least one porous filter element can have any suitable pore structure, e.g., a pore size (for example, as evidenced by bubble point, or by K_L as described in, for example, U.S. Patent No. 4,340,479), a pore rating, a pore diameter (e.g., when characterized using the modified OSU F2 test as described in, for example, U.S. Patent No. 4,925,572), that reduces or allows the passage therethrough of one or more materials of interest as the fluid is passed through the element. In some embodiments wherein the porous medium comprises a leukocyte depletion medium, while it is believed leukocytes are primarily removed by adsorption, they can also be removed by filtration. The pore structure can be selected to remove at least some level of leukocytes, while allowing the passing therethrough of desired components, e.g., at least one of plasma, platelets, and red blood

cells. The pore size or removal rating used depends on the composition of the fluid to be treated, and the desired effluent level of the treated fluid.

[0056] The porous medium/media and/or porous filter element(s) can have any desired critical wetting surface tension (CWST, as defined in, for example, U.S. Patent No. 4,925,572). Typically, the porous medium/element has a CWST of greater than about 53 dynes/cm (about 5.3 x 10^{-5} N/cm), more typically greater than about 58 dynes/cm (about 5.8 x 10^{-5} N/cm), and can have a CWST of about 66 dynes/cm (about 6.6 x 10^{-5} N/cm) or more. In some embodiments, the porous medium/element has a CWST of 75 dynes/cm (about 7.5 x 10^{-5} N/cm) or more. In some embodiments, the porous medium/element may have a CWST in the range from about 62 dynes/cm to about 115 dynes/cm (about 6.2 to about 16.2 x 10^{-5} N/cm), e.g., in the range of about 80 to about 100 dynes/cm (about 8.0 to about 10.0 x 10^{-5} N/cm). In some embodiments, the porous medium/element has a CWST of about 85 dynes/cm (8.5 x 10^{-5} N/cm), or greater, e.g., in the range from about 90 to about 105 dynes/cm (about 9.0 to about 10.5 x 10^{-5} N/cm), or in the range from about 85 dynes/cm to about 98 dynes/cm (about 8.5 to 9.8 x 10^{-5} N/cm).

[0057] The surface characteristics of the porous medium/element can be modified (e.g., to affect the CWST, to include a surface charge, e.g., a positive or negative charge, and/or to alter the polarity or hydrophilicity of the surface) by wet or dry oxidation, by coating or depositing a polymer on the surface, or by a grafting reaction. Modifications include, e.g., irradiation, a polar or charged monomer, coating and/or curing the surface with a charged polymer, and carrying out chemical modification to attach functional groups on the surface. Grafting reactions may be activated by exposure to an energy source such as gas plasma, heat, a Van der Graff generator, ultraviolet light, electron beam, or to various other forms of radiation, or by surface etching or deposition using a plasma treatment.

[0058] In accordance with preferred embodiments of the invention, at least one porous medium comprises a positively charged medium having a coating having fixed positive charges, preferably a crosslinked coating having fixed positive charges. In one embodiment, the medium includes a positively charged coating as described in International Publication No. WO 00/69549. In some embodiments, the one or more positively charged media have an isoelectric point of at least about 7, for example, 7.5 or more. In some embodiments, the isoelectric point is at least about 8, at least about 9, or at least about 10.

[0059] In an embodiment, the at least one porous medium can be a self-supporting medium.

[0060] At least one porous medium can comprise a porous fibrous medium or a porous membrane. Preferably, at least one porous medium comprises a positively charged medium. For example, at least one porous medium can comprise a positively charged porous fibrous medium including a crosslinked coating, or a porous membrane having a crosslinked coating. In those embodiments wherein the positively charged porous medium comprises a membrane including a porous substrate and a crosslinked coating, the porous substrate can be hydrophilic or hydrophobic.

[0061] In an embodiment, the coating has pendant cationic groups, i.e., positively charged groups that are not constrained by a polymer backbone or a crosslinked polymer network. Pendant cationic groups are groups that project from the polymer backbone, are bound to the polymer backbone by one covalent linkage, and are not constrained by additional linkages with the polymer backbone or the crosslinked polymer network. In some embodiments, pendant cationic groups are side-chains that project from the polymer backbone, and are not constrained by additional crosslinking reactions to the polymer backbone or the crosslinked polymer network.

[0062] In another embodiment, the positively charged groups are not pendant, e.g., they are in the polymer backbone. Embodiments of positively charged porous media having a crosslinked coating comprising pendant cationic groups (preferably, pendant quaternary ammonium groups) can be substantially free of non-pendant cationic groups, and embodiments of positively charged porous media having a crosslinked coating comprising non-pendant cationic groups can be substantially free of pendant cationic groups. In yet another embodiment, the crosslinked coating has pendant and non-pendant cationic groups (preferably, quaternary ammonium groups). In still another embodiment, the positively charged porous medium has a crosslinked coating comprising primary, secondary and/or tertiary amino groups, preferably, wherein the groups are present in the coating in a predetermined ratio, more preferably, a molar ratio of 1:2:1. However, aspects of the invention also include two or more combinations of any of the above-described embodiments of positively charged media with coatings. For example, an embodiment can include a first porous medium comprising a positively charged porous medium having a crosslinked coating comprising primary, secondary and tertiary amino groups; and a second porous medium comprising a positively charged porous medium having pendant and/or non-pendant positively charged quaternary ammonium groups.

[0063] In an embodiment, the crosslinked coating comprises a crosslinked polyamine such as a polyalkyleneamine, more preferably polyethyleneimine (PEI). For example, the crosslinked coating can comprise a polyethyleneimine having pendant positively charged groups. In some embodiments, the crosslinked coating comprises polyethyleneimine having pendant positively charged groups wherein the coating is substantially free of non-pendant positively charged groups.

[0064] The crosslinked coating can comprise a polyethyleneimine modified to contain positively charged groups, preferably, quaternary ammonium groups. The coating can include pendant and non-pendant quaternary ammonium groups. Illustratively, a modified polyethyleneimine can be prepared by reacting a polyethyleneimine with epichlorohydrin such that some or all of the tertiary amino groups on the epichlorohydrin are converted to quaternary ammonium groups. Such epichlorohydrin modified polyethyleneimines (that can be self crosslinking) can be obtained commercially. For example, LUPASOL™ SC-86X is an epichlorohydrin modified polyethyleneimine available from BASF Corporation in Mount Olive, NJ. Preferably, the coating further includes a polydimethylamine, e.g., a quaternized poly(dimethylamine-co-epichlorohydrin). One example of a suitable quaternized poly(dimethylamine-co-epichlorohydrin) is available as Catalog No. 652, Scientific Polymer Products, Inc., Ontario, NY.

[0065] In yet another embodiment, the coating comprises polyethyleneimine containing primary, secondary and/or tertiary amino groups, preferably, wherein the coating is substantially free of pendant quaternary ammonium groups. In some embodiments, the amino groups are in the coating in a predetermined ratio, more preferably, wherein the coating includes primary, secondary and tertiary groups, in some embodiments, wherein the primary, secondary and tertiary groups are present in the coating in a molar ratio of 1:2:1. Illustratively, the coating can be prepared by crosslinking polyethyleneimine (PEI) with polyethylene glycol diglycidylether (PEGDGE). The ratio of the amino groups can be controlled by a suitable choice of the PEI and the relative amounts of PEI and PEGDE.

[0066] In some embodiments, the coating can be prepared by crosslinking a composition comprising a polyethyleneimine and an amine reactive compound having a cationic group.

[0067] In accordance with embodiments of the invention, the coating, preferably, the crosslinked coating, can optionally include other materials such as ingredients, reactants, or polymers, e.g., copolymers. The other ingredients, reactants, or polymers can also include positively charged groups, including pendant positively charged groups. The present

invention further includes positively charged media having positively charged groups (including pendant and/or non-pendant positively charged groups) that can be produced by interchanging and/or combining the ingredients, reactants, or polymers of the embodiments described herein.

[0068] In one embodiment, the positively charged medium includes a crosslinked coating that includes a diallylamine copolymer. The present invention further provides a positively charged medium having a crosslinked coating that includes an acrylic copolymer. The pendant cationic groups are preferably linked to backbone of the crosslinked coating, particularly to the copolymer backbone, through spacer groups.

[0069] In some embodiments, the crosslinked coating is prepared by crosslinking a composition comprising a diallylamine copolymer having epoxy groups and pendant cationic groups, a polyamine such as a polyalkyleneamine, and an amine reactive compound having a cationic group. The crosslinked coating can also be prepared by crosslinking a composition comprising diallylamine, a diallyldialkylammonium halide, an acrylic monomer having a quaternary ammonium group, and a crosslinking agent.

[0070] In accordance with other embodiments of the invention, the medium has a crosslinked polyamine coating, particularly a crosslinked polyalkyleneamine coating, that provides a fixed positive charge. In other embodiments, the medium has a crosslinked coating comprising the reaction product of a polyethyleneimine having pendant quaternary ammonium groups and a polyalkyleneglycol polyglycidylether. The medium can include a crosslinked coating comprising a polyamine, such as a polyalkyleneamine, having pendant cationic groups.

[0071] The cationic group, that can be a pendant cationic group, can be any suitable cationic group - ammonium, sulfonium, phosphonium, or other group, preferably an ammonium group. An example of a preferred ammonium group is a quaternary ammonium group such as a tetraalkylammonium group.

[0072] Pendant cationic groups can be linked to the backbone directly through a bond such as a mono or divalent covalent bond, and the cationic groups can be linked and spaced away from the backbone of the coating. Spacer groups can be included between the backbone and the cationic groups to provide spatial charge separation and an increased opportunity for the fixed charges to interact with charged materials being treated. In some aspects of the invention, the spacer group can provide enhanced prion binding capacity and/or selectivity.

[0073] Any suitable spacer group can be employed, preferably a polar group such as a spacer group that includes a polar moiety. Thus, for example, the spacer group includes one or more moieties selected from the group consisting of hydroxy, hydroxyalkyl, amino, imino, aminoalkyl, amido, alkylamido, urea, urethane, ester, and alkoxyalkyl.

[0074] In certain embodiments, the spacer group includes one or more moieties selected from the group consisting of hydroxyalkyl, alkylamino, hydroxyalkylaminoalkyl, hydroxyalkylaminoalkyl hydroxyalkyl, alkylaminoalkyl, and alkylamido. In certain other embodiments, the spacer group includes one or more moieties selected from the group consisting of hydroxyalkyl, alkylamino, hydroxyalkylaminoalkyl, hydroxyalkylaminoalkyl hydroxyalkyl, alkylaminoalkyl, and alkylamido.

[0075] The spacer group can be linear, branched, or combinations thereof. The cationic groups can become integral parts of the coating as a result of attachment of the cationic groups with the aid of reactants that link the cationic groups to the backbone of the coating through spacer groups. In certain embodiments, the cationic groups can be attached to precursors through spacer groups and the precursors can be converted to or become part of the coating chemistry.

[0076] The spacer group can of any suitable length, for example, the spacer group can be a group having from 1 to about 10 atoms, e.g., carbon atoms. Thus, the spacer group can be from 1 to about 10 carbon atoms long, preferably from 2 to about 6 carbon atoms long, and more preferably about 3 carbon atoms long. Without intending to be bound to any particular mechanism, the spacer group, which helps provide spatial separation among the cationic groups, is believed to have a role in enhancing the interaction between the prions and the porous medium and in the binding capacity that is manifested as a result of that interaction.

[0077] One preferred spacer group is hydroxyalkyl. Thus, e.g., the polyamine can be contacted with a glycidyl compound having a cationic group so that the epoxy ring opens at the primary or secondary amino groups of the polyalkyleneamine. Further, a solution of a polyamine such as polyethyleneimine (PEI) can be combined with, e.g., glycidyl trimethylammonium chloride, and the polyamine having trimethylammonium chloride pendant groups linked through hydroxyalkyl groups can be obtained.

[0078] The quantities of the reactants for the linking reaction according to some embodiments of the invention are chosen so that the resulting polyamine having pendant cationic groups contains reactive sites for crosslinking. Thus, for example, the polyamine contains residual primary and/or secondary amino groups after the linking reaction has been

carried out. Accordingly, it is preferred that the residual primary and/or secondary amino groups are at least about 10%, and more preferably from about 10% to about 40%, by mole, of the primary and/or secondary amino groups on the polyamine prior to linking of the pendant cationic groups.

[0079] The embodiment that includes a coating comprising a diallylamine copolymer can be further described as follows. The copolymer comprises polymerized diallylamine monomer. The diallylamine monomer can be substituted or unsubstituted. Thus, for example, an alkyl substituted diallylamine such as diallylmethylamine can be used. Preferably, the diallylamine copolymer further includes an acrylic monomer. The acrylic monomer preferably contains the cationic group. The acrylic monomer could be an acrylate ester or an acrylamide. The term "acrylic" herein includes unsubstituted as well as substituted acrylic monomers. An example of a substituted acrylic monomer is an alkylacrylic monomer.

[0080] The term "alkyl" herein refers to an alkyl group having 1 to about 10 carbon atoms, preferably from 1 to about 6 carbon atoms.

[0081] An example of a suitable acrylamide monomer is an acryloylaminoalkyl monomer. An example of the acryloylaminoalkyl monomer is methacryloylaminopropyl trimethylammonium chloride. An example of an acrylate is an acryloyloxyalkyl monomer.

[0082] In certain embodiments of the present invention, the diallylamine copolymer includes one or more comonomers. These comonomers are preferably nitrogen containing monomers. These comonomers preferably contain tertiary and/or quaternary ammonium groups. Examples of preferred comonomers include diallyldialkylammonium chloride and dialkylaminoalkyl acrylamide. Thus, in certain embodiments, the diallylamine copolymer includes in addition to diallylamine, comonomers such as methacryloylaminopropyl trimethylammonium chloride, diallyldimethylammonium chloride, and dimethylaminopropyl methacrylamide. The acrylic copolymer can also include an acrylate monomer in place of, or in addition to, the acrylamide monomer.

[0083] The diallylamine copolymer can be prepared by methods known to those of ordinary skill in the art. For example, diallylamine and monomers carrying cationic and epoxy groups can be polymerized to provide a suitable diallylamine copolymer.

[0084] Alternatively, a diallylamine copolymer that contains cationic groups can be prepared first, and then, the epoxy groups can be introduced. Thus, e.g., the copolymer can be reacted with a reactive epoxy group containing compound, e.g., epichlorohydrin.

[0085] A mixture of diallylamine and the acrylic monomer is allowed to polymerize under suitable conditions. For example, a free radical initiator such as ammonium persulfate can be employed to initiate the polymerization. The molar ratio between diallylamine and the acrylic monomer can be from about 0.05 to about 4, preferably from about 0.1 to about 2, and more preferably from about 0.5 to about 1.

[0086] The diallylamine copolymer is then reacted with epichlorohydrin. The chloromethyl group of epichlorohydrin reacts with the secondary or primary amino groups of the copolymer to provide a polymer having pendant epoxy groups. The degree of substitution of epoxy groups can be from about 10% to about 200%, preferably from about 25% to about 175%, and more preferably from about 50% to about 150%, by mole of the diallylamine in the copolymer.

[0087] Some of the cationic groups of the coating can become part of the coating chemistry by attaching them through amino spacer groups. Amino spacer groups can be provided by the use of one or more polyamines such as polyalkyleneamines.

[0088] In accordance with embodiments of the invention, examples of polyalkyleneamines include short chain polyalkyleneamines such as diethylenetriamine, triethylenetetramine, tertaethylenepentamine, and pentaethylenehexamine, and the like, and long chain polyalkylenamines, such as polyethyleneimine (PEI). The polyamines have a molecular weight of greater than about 500. Any suitable polyamine amine can be used. In preferred embodiments of the invention, a PEI is used. Any suitable PEI can be used. The molecular weight of the PEI can be from about 500 to about 750,000, preferably from about 10,000 to about 750,000, and more preferably from about 50,000 to about 100,000. In certain embodiments of the present invention, a combination of a short chain polyalkyleneamine and a long chain polyalkyleneamine can be used advantageously.

[0089] The cationic groups can be attached to the polyalkyleneamine through suitable spacer groups. For example, the polyalkyleneamine can be reacted with a glycidyl compound bearing a cationic group, e.g., glycidyl trialkylammonium halide, to provide a polyalkyleneamine having cationic groups linked to the polyalkyleneamine through hydroxyalkyl spacer groups. Thus, for example, pentaethylenehexamine can be reacted with glycidyl trimethylammonium chloride.

[0090] The polyalkyleneamine that is modified to have cationic groups as described above can be made part of the coating chemistry by reacting it with the diallylamine copolymer having suitable reactive sites, e.g., epoxy groups. The diallylamine copolymers

can be made to possess reactive sites by reacting with compounds that provide reactive sites. Thus, for example, the diallylamine copolymer can be reacted with epichlorohydrin to provide epoxy sites. The coating is crosslinked as a result of reaction among the reactive sites such as epoxy and amine groups. In some embodiments of the present invention, the coating further includes a crosslinking agent. Any suitable crosslinking agent can be used. The crosslinking agent is preferably a polyglycidyl compound, for example, a polyalkyleneglycol polyglycidylether. A preferred example of a crosslinking agent is ethylene glycol diglycidyl ether.

[0091] Another embodiment of the present invention further provides a positively charged porous medium having a crosslinked polyalkyleneamine coating, that provides a fixed positive charge.

[0092] In another embodiment, the crosslinked coating comprises the reaction product of a polyethyleneimine having pendant quaternary ammonium groups and a polyalkyleneglycol polyglycidylether.

[0093] In an embodiment, the coating can be created by coating and curing a fibrous web or a hydrophilic substrate with a composition comprising, in suitable amounts, a diallylamine copolymer, a polyamine such as a polyalkyleneamine, and an amine reactive agent having a cationic group, such as glycidyl trimethylammonium chloride. For example, the diallylamine copolymer can be present in an amount of from about 1% to about 20%, preferably in an amount of from about 2% to about 10%, and more preferably in an amount of from about 3% to about 7% by weight of the composition. The polyamine can be present in an amount of from about 0.05% to about 5%, preferably in an amount of from about 0.1% to about 2%, and more preferably in an amount of from about 0.2% to about 1% by weight of the composition. The ratio of amine reactive cationic compound and active hydrogen of polyalkyleneamine can be present in an amount of from about 0.1% to about 20%, preferably in an amount of from about 0.5% to about 15%, and more preferably in an amount of from about 0.75% to about 10% by weight of the composition.

[0094] Certain embodiments of a positively charged porous medium according to the present invention can be prepared by coating and curing a hydrophilic substrate or a fibrous web with a coating composition comprising diallylamine, diallyldimethylammonium halide, an acrylic monomer, and a crosslinking agent. An example of a suitable diallyldialkylammonium halide is diallyldimethylammonium chloride. Any suitable crosslinking agent can be used. A preferred crosslinking agent is an N-(alkoxymethyl)-

acrylamide. The crosslinking acrylamide can be further substituted. For example, an alkylacrylamide having N-(alkoxymethyl) group can be used. Preferred crosslinking agents are N-(isobutoxymethyl)acrylamide and N-(isobutoxymethyl)methacrylamide. The ingredients of the composition can be in any suitable proportion.

[0095] For example, the acrylic monomer can be present in an amount of from about 0.1% to about 30%, preferably in an amount of from about 1% to about 20%, and more preferably in an amount of from about 1% to about 15% by weight of the composition. The diallylamine can be present in an amount of from about 0.1% to about 30%, preferably in an amount of from about 1% to about 20%, and more preferably in an amount of from about 1% to about 15% by weight of the composition. The diallylamine dialkylammonium halide can be present in an amount of from about 0.1% to about 30%, preferably in an amount of from about 1% to about 20%, and more preferably in an amount of from about 2% to about 15% by weight of the composition. The crosslinking agent can be present in an amount of from about 0.1% to about 20%, preferably in an amount of from about 1% to about 15%, and more preferably in an amount of from about 2% to about 10% by weight of the composition.

[0096] In certain embodiments of the present invention, the coating comprises an acrylic copolymer having pendant cationic groups linked to the copolymer through spacer groups. Any of the spacer groups described above can be utilized. An example of the acrylic monomer is alkylacryloylaminoalkyl trialkylammonium halide, preferably methacryloylaminopropyl trimethylammonium chloride. Thus, the coating composition includes an acrylic copolymer that has epoxy groups and pendant cationic groups. The acrylic copolymer comprises a glycidylalkylacrylate and a methacryloyloxyalkyl or methacryloylaminoalkyl trialkylammonium halide, and preferably glycidyl methacrylate and methacryloyloxyethyl or methacryloylaminopropyl trimethylammonium chloride. It is further preferred that the acrylic copolymer in its crosslinked state includes a polyamine. A short chain polyalkyleneamine is a further preferred polyamine.

[0097] Illustratively, the acrylic copolymer in a crosslinked state includes pentaethylenehexamine. Preferably, the polyamine is a modified polyamine. Thus, the polyamine is modified by a compound having cationic groups. The polyamine is modified by glycidyl trimethylammonium chloride. The coating composition can include the ingredients in any suitable proportion. For example, the acrylic polymer can be present in an amount of from about 0.5% to about 5%, preferably in an amount of from about 1% to about 4.5%, and more preferably in an amount of from about 1.25% to about 4.25% by weight of the

composition. The polyamine can be present in an amount of from about 0.5% to about 10%, preferably in an amount of from about 1% to about 8%, and more preferably in an amount of from about 3% to about 6% by weight of the composition.

[0098] A polyamine having cationic groups can be crosslinked by any suitable crosslinking agent. The crosslinking agent is a polyfunctional agent having amine reactive groups such as epoxy, isocyanato, carboxyl, and acid chloride. A preferred crosslinking agent is a polyglycidyl compound. An example of a suitable polyglycidyl compound is a polyalkyleneglycol polyglycidylether. Ethyleneglycol diglycidyl ether and butyleneglycol diglycidyl ether are preferred crosslinking agents.

[0099] In an embodiment, polyethyleneimine (PEI) is crosslinked by polyethyleneglycol diglycidyl ether (PEGDGE), preferably to provide a coating substantially free of quaternary amino groups. The crosslinked coating can contain primary, secondary and tertiary amino groups, preferably, in a desired ratio, e.g., in a primary to secondary to tertiary molar ratio of 1:2:1. The ratio of such amino groups can be controlled by, for example, a suitable choice of the PEI and the relative amounts of PEI and PEGDGE, and the pH of the polymer solution during crosslinking.

[0100] PEI can be linear or preferably branched. An example of a branched PEI is of the formula $(-NHCH_2CH_2)_x-[N(CH_2CH_2NH_2)CH_2CH_2-]_y$, wherein x+y = 1, and x and y can be varied independently, e.g., from about 0.01 to 1, preferably from about 0.1 to about 0.9, and more preferably from about 0.3 to about 0.7. During the reaction between PEI and PEGDGE, some of the amino groups react with the glycidyl groups of PEGDGE to create a crosslinked coating. The pH of the solution is preferably controlled to minimize the formation of quaternary amino groups. PEI and PEGDGE can be used (as starting materials) in any suitable amount or proportion, e.g., in a molar ratio of from about 0.001:1 to about 1:0.001, preferably from about 0.074:1 to about 1:0.004, more preferably, from about 0.68:1 to about 1:0.034. The pH of the coating solution can be controlled by the addition of an acid, e.g., hydrochloric acid, to provide a pH in the range of from about 7 to about 11, preferably, in the range of from about 8 to about 10, more preferably, in the range of from about 8.3 to about 8.7

[0101] The coating composition can be prepared, for example, by dissolving the polyamine in a suitable solvent. Preferred solvents include water, low boiling alcohols such as methanol, ethanol, and propanol, and combinations thereof. The solvent can be present in an amount of from about 40% to about 99%, and preferably in an amount of from about 90% to about 99% by

weight of the coating composition. The polyamine can be present in an amount of from about 1% to about 5%, and preferably in an amount of from about 1% to about 2.5% by weight of the coating composition.

[0102] The positively charged media according to the invention can be made by coating a fibrous web (e.g., a fibrous woven web or, more preferably, a fibrous non-woven web) or a porous substrate, (preferably, a porous hydrophilic substrate), and curing the coated web or substrate.

[0103] The fibrous web or porous substrate can be made of any suitable material; preferably, the web or substrate comprises a polymer. A variety of polymers are suitable.

[0104] Suitable polymers for substrates include, for example, polyaromatics, polysulfones, polyamides, polyimides, polyolefins, polystyrenes, polycarbonates, cellulosic polymers such as cellulose acetates and cellulose nitrates, fluoropolymers, and PEEK. Aromatic polysulfones are preferred. Examples of aromatic polysulfones include polyethersulfone, bisphenol A polysulfone, and polyphenylsulfone. The hydrophilic porous substrate can have any suitable pore size, for example, a pore size of from about 0.01 or 0.03 μm to about 10 μm, about 0.1 μm to about 5 μm, and from about 0.2 μm to about 5 μm. The porous substrate can be asymmetric, or symmetric.

[0105] The fibrous web and porous substrate can be prepared by methods known to those of ordinary skill in the art. For example, the porous substrate can be prepared by a phase inversion process. Thus, a casting solution containing the polymer, a solvent, a pore former, a wetting agent, and optionally a small quantity of a non-solvent is prepared by combining and mixing the ingredients, preferably at an elevated temperature. The resulting solution is filtered to remove any impurities. The casting solution is cast or extruded in the form of a sheet or hollow fiber. The resulting sheet or fiber is allowed to set or gel as a phase inverted membrane. The set membrane is then leached to remove the solvent and other soluble ingredients.

[0106] Suitable polymers, preferably, synthetic polymers, for producing fibrous webs include, for example, polybutylene terephthalate (PBT), polyethylene, polyethylene terephthalate (PET), polypropylene, polymethylpentene, polyvinylidene fluoride, sulfones (e.g., aromatic sulfones such as polysulfone, polyethersulfone, and polyarylsulfone), and polyamides such nylon 6, nylon 66, nylon 6T, nylon 612, nylon 11, and nylon 6 copolymers.

[0107] The fibrous webs, that can comprise a woven web or a non-woven web, can be prepared as known to those of ordinary skill in the art. Preferably, the fibrous non-woven

web, more preferably, a fibrous non-woven synthetic polymeric web, can be prepared by melt-blowing, as disclosed in, for example, U.S. Patent Nos. 4,880,548; 4,925,572, and 6,074,869.

[0108] If desired, the filter/filter element(s)/porous medium/media can be non-releasably sealed in the housing to provide the prion capture device. For example, the housing can be sealed utilizing, for example, an adhesive, a solvent, radio frequency sealing, ultrasonic sealing and/or heat sealing. Additionally, or alternatively, the housing can be sealed via injection molding.

[0109] Alternatively, the filter/filter element/porous medium can be releasably sealed in the housing to provide the prion capture device. For example, the housing can comprise a jig or swinney device, wherein the filter/filter element/porous medium is fluid-tightly sealed in the housing during use, and the jig/swinney device can be opened so that the filter/filter element/porous medium can be removed from the housing. In one embodiment, the filter/filter element/porous medium is removed, placed in pathogenic conformer releasing solution, and agitated, e.g., vortexed, to release the prions.

[0110] Any housing of suitable shape to provide, for example, at least one inlet and at least one outlet, at least one inlet and at least first and second outlets, or to provide a plurality of wells, may be employed. The housing can be fabricated from any suitable rigid impervious material, including any impervious thermoplastic material, which is compatible with the biological fluid being processed. For example, the housing can be fabricated from a metal, such as stainless steel, or from a polymer. In an embodiment, the housing is a polymer, for example, a transparent or translucent polymer, such as an acrylic, polypropylene, polystyrene, or a polycarbonated resin. Such a housing is easily and economically fabricated, and allows observation of the passage of the fluid through the housing. The housing may include an arrangement of one or more channels, grooves, conduits, passages, ribs, or the like, which may be serpentine, parallel, curved, circular, or a variety of other configurations.

[0111] Figure 1 (1A-1C) illustrates one embodiment of a capture device or a capture/release device 100 according to the invention comprising a housing 50 including an inlet section 10 and an outlet section 20. In the exploded view shown in Figure 1C, the inlet section and the outlet section include, respectively, in the portions facing the upstream and downstream surface of the prion capture (or capture/release) filter 5, generally annular ribs 11 and 21, (in this illustrated embodiment the ribs are not continuous) and generally annular

flow channels 12 and 22, as well as radial flow channels 13 and 23 intersecting with the annular flow channels.

[0112] In some embodiments, the inlet and outlet sections of the housing include different structures. For example, Figure 2 illustrates another embodiment of a capture device or a capture/release device 100 according to the invention comprising a housing 50 including an inlet section 10 and an outlet section 20. Figure 2A shows an exploded view, wherein the inlet section 10 includes, in the portion facing the upstream surface of the filter 5, generally radial ribs 15. In this illustrated embodiment, the ribs have an alternating structure, with some ribs (15a) extending substantially across the diameter of the inner face of the housing, and the other ribs (15b) extending approximately one-quarter to one-third of the diameter of the inner face 18 of the housing. As shown in more detail in Figure 2B, the inner portion of this illustrated embodiment of the inlet section has a generally domed area 19. In those embodiments wherein the inlet and/or outlet portion includes a domed area, the filter can be deflected into that area during flushing while minimizing contact with the housing.

[0113] Figure 2A also shows the outlet section 20, wherein the outlet section includes, in the portion facing the downstream surface of the filter, generally annular ribs 21 (in this illustrated embodiment the ribs are not continuous) and generally annular flow channels 22, as well as radial flow channels 23 intersecting with some of the annular flow channels.

[0114] In some embodiments, the ribs and channels provide for support and/or drainage, e.g., during forward flow and/or reverse flow.

[0115] In one embodiment, the prion capture device or prion capture/release device is adapted to provide quasi-static flow through the device.

[0116] In some embodiments of the prion capture device or the prion capture and release device, it is desirable to provide equivalent or substantially equivalent unit flux over the medium/media surface, while minimizing resistance or channels that could cause localized differences in flux through the medium/media during prion capture and/or release. Alternatively, or additionally, in some embodiments it is desirable to minimize the contact area of the housing structures with the total porous medium/media downstream surface area and/or upstream surface area.

[0117] The following examples further illustrate the invention but, of course, should not be construed as in any way limiting its scope.

EXAMPLE 1

[0118] This example demonstrates that prions can be removed from prion-containing whole blood, plasma, and phosphate buffered saline in accordance with embodiments of the invention. 47 mm diameter fibrous non-woven web discs of 12 layers of melt-blown polybutylene terephthalate fibers are obtained to provide filter elements. Each layer has a basis weight of 5.2 g/ft2, and a thickness of about 16 mil, and the fibers have an average diameter of 3.8 micrometers. The 12 layer disc has a thickness of about 192 mil.

[0119] One set of discs is coated as follows:

[0120] A coating solution is prepared from the following ingredients.

Ingredients	Wt.%
DI Water	83.23
6N Hydrochloric Acid	4.8
Branched PEI (Aldrich Chemical Company), avg. Mw=50,000 - 100,000, 30% solids	11.67
Polyethyleneglycol (600) diglycidyl ether	0.3

[0121] The coating solution is prepared by combining and mixing the above ingredients for about 1 hour. The pH of the solution is 8.5. The fibrous web discs are dipped in the coating solution, and the coated web discs are then cured at a temperature of about 100°C for 30 minutes. The cured web discs are extracted in hot deionized water and dried in air to provide filter elements with positively charged crosslinked coatings.

[0122] A second set of discs is coated as follows:

[0123] A coating solution is prepared from the following ingredients.

Ingredients	Wt.%
DI Water	94.5
Potassium hydroxide	1.0
Epichlorohydrin modified polyethyleneimine	2.0
Quaternized poly(dimethylamine-co-epichlorohydrin)	2.5

[0124] The coating solution is prepared by combining and mixing the above ingredients for about 1 hour. The pH of the solution is adjusted to approximately 10.6 using a 20%

potassium hydroxide solution immediately before use. The fibrous web discs are dipped in the coating solution, and the coated web discs are cured at a temperature of 120°C for 30 minutes. The cured web discs are extracted in hot deionized water and dried in air to provide filter elements with positively charged crosslinked coatings.

[0125] A third set of discs is coated as follows:

[0126] 4.8 grams of a 50% solution of branched PEI from Aldrich Chemical Company having an average molecular weight of 50,000 – 100,000 are combined with 9.5 grams of a 90% solution in water of glycidyl trimethylammonium chloride (Fluka) and 184.7 grams of deionized water. The resulting solution is agitated for about 15 minutes and then placed in a water bath at 36°C for 24 hours to obtain a solution containing a modified PEI. 1.0 gram of a 50% solution of ethyleneglycol diglycidylether (Aldrich) is mixed with the solution containing the modified PEI prepared above to obtain a coating solution. The fibrous web discs are dipped in the coating solution. The coated webs are cured at a temperature of 120°C for 30 minutes. The cured web discs are extracted in hot deionized water and dried in air to provide filter elements with positively charged crosslinked coatings.

[0127] Each filter element is placed in a polycarbonate housing before use.

[0128] Brain samples from scrapie-infected hamsters are obtained, homogenized, solubilized, and spiked (1:10) with CP2D anticoagulated whole blood (stored at room temperature for about 5 hours), plasma, or phosphate buffered saline at final concentration of 1% (approximately 10 mg/mL of infectious scrapie brain homogenate). A filter head height of 30 inches (about 76.2 cm) is provided, and twenty milliliters of each sample is passed through a separate filter element at a flow rate of about 2-3 ml per minute.

[0129] The concentration of infectious prions is determined before and after filtration using a Western blot (using 3F4 and 7G5 monoclonal antibodies), with and without proteinase K digestion (normal proteins are digested or degraded by proteinase K, and infectious prions are resistant to proteinase K digestion). Additionally, hemoglobin levels are determined, and leukocyte and platelet counts are taken before and after filtration, and the presence of plasma proteins before and after filtration is determined via Coomassie staining.

[0130] Analysis of the results shows the infectious and non-infectious prions are removed with 100% efficiency from the various fluid samples. A Western blot of unfiltered non-proteinase K treated spiked fluid shows bands of proteins at 34kD and 27kD, a Western blot of unfiltered spiked fluid treated with proteinase K shows a band of proteins at 27kD, and a Western blot of unfiltered normal hamster brain homogenate treated with proteinase K

does not show bands of proteins at 27kD and 34kD. The Western blots of the filtered proteinase K and non-proteinase K treated spiked fluids show no bands of proteins at 27kD and 34kD.

[0131] Additionally, the plasma proteins in the plasma and whole blood, and the red blood cells in the whole blood, are not adversely affected by the treatment. For example, the filtered red blood cells have hemoglobin levels of about 5 mg/dl (far below the European standard of no more than 0.8% hemolysis, corresponding to a hemoglobin level of less than 200 mg/dl). The filter element removes over 99.9% of the platelets in the whole blood, and over 99.9% of the leukocytes in the whole blood and the plasma. Coomassie staining shows the same plasma proteins are present in the unfiltered and filtered fluid.

EXAMPLE 2

[0132] This example demonstrates that non-cell associated (non-leukocyte associated) prions can be removed from prion-containing packed red blood cells in accordance with a filter element according to the present invention.

[0133] Two forms of filters comprising two sets of filter elements having different surface chemistries are prepared. One set of filter elements has positively charged fibrous media, the other set of elements has negatively charged fibrous leukocyte depletion media.

[0134] One form of filters has the two sets of each of the positively charged and negatively charged filter elements in an alternating arrangement, the other form of filters has a negatively charged filter element followed by a positively charged element.

[0135] Positively charged porous fibrous media are prepared as follows:

[0136] Two 47 mm diameter fibrous non-woven web discs of 2 layers of melt-blown polybutylene terephthalate fibers, and one 47 mm diameter fibrous non-woven web disc of 4 layers of melt-blown polybutylene terephthalate fibers are obtained. Each layer has a basis weight of 5.2 g/ft^2, and a thickness of about 16 mil, and the fibers have an average diameter of 3.8 micrometers.

[0137] A coating solution is prepared from the following ingredients.

Ingredients	Wt.%
DI Water	94.5
Potassium hydroxide	1.0
Epichlorohydrin modified polyethyleneimine	2.0

Quaternized poly(dimethylamine-co-epichlorohydrin)	2.5

[0138] The coating solution is prepared by combining and mixing the above ingredients for about 1 hour. The pH of the solution is adjusted to approximately 10.6 using a 20% potassium hydroxide solution immediately before use. The fibrous web disc is dipped in the coating solution, and the coated web disc is cured at a temperature of 120°C for 30 minutes. The cured web disc is extracted in hot deionized water and dried in air to provide a fibrous porous filter element with a positively charged crosslinked coating.

[0139] The positively charged media have CWSTs of about 97 dynes/cm.

[0140] Negatively charged porous fibrous leukocyte depletion media are prepared as follows:

[0141] Two 47 mm diameter fibrous non-woven web discs of 4 layers of melt-blown polybutylene terephthalate fibers, and one 47 mm diameter fibrous non-woven web disc of 8 layers of melt-blown polybutylene terephthalate fibers, are obtained. Each layer has a basis weight of 5.2 g/ft^2, and a thickness of about 16 mil, and the fibers have an average diameter of 3.8 micrometers.

[0142] The fibrous leukocyte depletion media are surface grafted as disclosed in U.S. Patent No. 4,925,572. They have a negative zeta potential of about -22 millivolts at a pH of about 7.2. They also have CWSTs of about 68 ($\pm$3) dynes/cm.

[0143] The filter elements are arranged in a jig as follows:

[0144] In an alternating arrangement wherein the first filter element (nearest the inlet) is a positively charged filter element having 2 layers, followed by a second filter element, which is a leukocyte depletion element having 4 layers, followed by another positively charged filter element having 2 layers, and the final element (nearest the outlet) is another leukocyte depletion element having 4 layers.

[0145] In another arrangement wherein the first filter element (nearest the inlet) is a leukocyte depletion element having 8 layers, followed by a positively charged filter element having 4 layers.

[0146] A prefilter element is also placed in each jig, wherein the prefilter element is upstream of the first filter element.

[0147] Units of whole blood are obtained from healthy volunteers and collected in bags containing CPD anticoagulant. Red blood cell concentrates are prepared in accordance with standard blood bank practice, resuspended in SAGM additive solution, and filtered using a

leukocyte depletion filter (BPF4™ High Efficiency Filter, Pall Corp. NY) to provide leukocyte-depleted red cell concentrates. Four units of ABO/Rh compatible concentrates are pooled together to provide a homogenous pool of about 1200 mL. 100 mL of 10% scrapie infected hamster brain homogenates are added to 900 mL of the red cell concentrates such that the final concentration of brain homogenate is reduced to 1%. The scrapie-contaminated red cell concentrates are divided into 300 mL aliquots, and the aliquots are filtered with another BPF4™ High Efficiency Filter leukocyte filter, or a filter including two sets of elements with different surface chemistries as described above. The concentration of infectious proteins in the red cell concentrates before and after filtration is measured using Western blot assays, conducted with and without proteinase K.

[0148] The results show that the filters including a positively charged filter element and a leukocyte depletion element more effectively remove infectious prions from the hamster prion-containing red cell concentrates than a filter including leukocyte depletion element by itself. This also shows that the positively charged filter element removes non-cell associated prions from the concentrates.

EXAMPLE 3

[0149] This example demonstrates pathogenic prions are captured by, and released from, an embodiment of a prion capture/release device according to an embodiment of the invention.

[0150] Two 13 mm diameter fibrous non-woven web filters, each having 6 layers of melt-blown polybutylene terephthalate fibers are obtained. The filters are treated to provide a positively charged coating as described in Example 2. Each filter has a physiological pH of greater than 11.

[0151] The filters are placed in housings to provide a prion capture/release device 100 and arranged in a system as shown in Figure 3. The system includes the prion capture/release device 100, a syringe for the prion-containing plasma (syringe barrel 3 shown), a syringe for the wash fluid (syringe barrel 2 shown), and a valve 75 for changing fluid flow paths to include one syringe or the other.

[0152] 25 ml of human CPDA plasma is spiked with 50 μl of 10% (v/v) Weanling Syrian Hamster's Brain Homogenate (SIHBH), and placed into a 50 ml tube. A 10 ml sample (pH 7 to 7.6; 22 ± 2°C) of the spiked plasma is filtered through each of the prion capture/release devices at a flow rate of 1 ml/min. The filtrates are collected in graduated cylinder 200.

Each filter is washed with 5 ml of PBS that is passed through the device at a flow rate of 1 ml/min, and the wash buffer is collected.

[0153] Subsequently, to elute the prions, 2 ml of 0.1% sarkosyl in PBS is passed through each of the filters in the opposite direction using a syringe.

[0154] 0.5 ml of each of the obtained eluted materials is mixed with 1.0 ml of 4% sarkosyl in PBS. Each mixture is then incubated at 37°C for 10 min. Following incubation, 122 μl of 4% PTA (Sigma PTA Sodium Salt, P-6395) in 170 mM $MgCl_2$ at pH 7.4 is added to each mixture. The resulting mixtures are each incubated with continuous mixing at 37°C for 30 minutes. Each mixture is centrifuged at 18,000 g for 60 minutes at room temperature. After centrifugation the supernatants are discarded and the pellets obtained are resuspended in 500 μl of 0.1% sarkosyl in PBS. The samples are centrifuged a second time at 18,000 g for 60 minutes at room temperature, and the supernatants are removed. The pellets obtained are resuspended in 40 μl of 0.1% sarkosyl in PBS with sonicating for 20-25 sec/sample. After the pellets are resuspended, 10 μl of 250 μg/ml pK is added to the 40 μl samples. The 50 μl samples (containing eluted prions) are then incubated at 52°C for 30 minutes.

[0155] Additionally, 0.5 ml of each of the obtained filtrates is mixed with 1.0 ml of 4% sarkosyl in PBS. Each mixture is then incubated at 37°C for 10 min. Following incubation, 122 μl of 4% PTA (Sigma PTA Sodium Salt, P-6395) in 170 mM $MgCl_2$ at pH 7.4 is added to each mixture. The resulting mixtures are each incubated with continuous mixing at 37°C for 30 minutes. Each mixture is centrifuged at 18,000 g for 60 minutes at room temperature. After centrifugation the supernatants are discarded and the pellets obtained are resuspended in 500 μl of 0.1% sarkosyl in PBS. The samples are centrifuged a second time at 18,000 g for 60 minutes at room temperature, and the supernatants are removed. The pellets obtained are resuspended in 40 μl of 0.1% sarkosyl in PBS with sonicating for 20-25 sec/sample. After the pellets are resuspended, 10 μl of 250 μg/ml pK is added to the 40 μl samples. The 50 μl filtrate samples are then incubated at 52°C for 30 minutes.

[0156] Additionally, several control samples are prepared with and without PTA.

[0157] With respect to the control sample prepared with PTA, 0.5 ml of 0.1% sarkosyl in PBS is spiked with 67 μl (75:1 v/v) of SIHBH. 0.5 ml of the spiked buffer is mixed with 1.0 ml of 4% sarkosyl in PBS. The mixture is then incubated at 37°C for 10 min. Following incubation, 122 μl of 4% PTA (Sigma PTA Sodium Salt, P-6395) in 170 mM $MgCl_2$ at pH 7.4 is added to the mixture. The resulting mixture is incubated with continuous mixing at

37°C for 30 minutes, and centrifuged at 18,000 g for 60 minutes at room temperature. After centrifugation the supernatant is discarded and the pellet obtained is resuspended in 500 μl of 0.1% sarkosyl in PBS. The sample is centrifuged a second time at 18,000 g for 60 minutes at room temperature, and the supernatant is removed. The pellet obtained is resuspended in 40 μl of 0.1% sarkosyl in PBS with sonicating for 20-25 sec. After the pellet is resuspended, 10 μl of 250 μg/ml pK is added to the 40 μl sample. The 50 μl sample is then incubated at 52°C for 30 minutes.

[0158] With respect to the control samples prepared without PTA, 1.0 ml samples of 0.1% sarkosyl in PBS are spiked with 6.7 μl, 20 μl, and 200 μl of SIHBH, to provide 1/150, 1/50, and 1/5 dilutions of SIHBH. The spiked control samples are resuspended in 40 μl of 0.1% sarkosyl in PBS, and sonicated for 20-25 sec/sample. 10 μl of 250 μg/ml pK is added to the samples, and the 50 μl samples are then incubated at 52°C for 30 minutes.

[0159] A spiked plasma control is also prepared. 0.5 ml of plasma is spiked with 1.33 μl of SIHBH (1:375 v/v), and mixed with 1.0 ml of 4% sarkosyl in PBS. The spiked plasma is then incubated at 37°C for 10 min. Following incubation, 122 μl of 4% PTA (Sigma PTA Sodium Salt, P-6395) in 170 mM $MgCl_2$ at pH 7.4 is added to the mixture. The resulting mixture is incubated with continuous mixing at 37°C for 30 minutes. The mixture is centrifuged at 18,000 g for 60 minutes at room temperature. After centrifugation the supernatant is discarded and the pellet obtained is resuspended in 500 μl of 0.1% sarkosyl in PBS. The sample is centrifuged a second time at 18,000 g for 60 minutes at room temperature, and the supernatant is removed. The pellet obtained are resuspended in 40 μl of 0.1% sarkosyl in PBS with sonicating for 20-25 sec. After the pellet is resuspended, 10 μl of 250 μg/ml pK is added to the 40 μl sample. The 50 μl plasma sample is then incubated at 52°C for 30 minutes.

[0160] A Western blot is performed. 17 μl of loading buffer (Invitrogen NuPAGE LDS Sample buffer, NP007) is added to 50 μl of each of the samples, which are then incubated for 10 minutes at 75-85°C. Next 20 μl of each sample is loaded into an Invitrogen Novex 4-12% Bis-Tris Gel and run for 30 minutes at 200 volts. The gel is transferred using Pall-Gelman Biotrace PVDF membrane at 25 volts and 130 mA for 1 hour. The membrane is blocked with 5% dry non-fat milk in PBS with 0.2% Tween-20 and is incubated for 30 minutes at room temperature.

[0161] 3F4 anti-PrP diluted 1:5000 in 5% dry non-fat milk in PBS with 0.2% Tween-20 is added as a primary antibody. The membrane is incubated for 2 hours at room temperature with shaking. The membrane is then washed for 5 minutes using PBS with 0.2% Tween-20. The washing of the membrane is repeated two more times.

[0162] A secondary antibody (Sigma goat anti-mouse to whole molecule HRP-IgG) is added at 1:2000 in 5% dry non-fat milk in PBS with 0.2% Tween-20. The membrane is incubated for 1 hour at room temperature. Following incubation, the membrane is once again washed 3 times for 5 minutes using PBS with 0.2% Tween-20.

[0163] The membrane is developed using PIERCE Super Signal West Dura Extended Duration Substrate (product #34076). The resulting Western blot shows the presence of prions in the spiked plasma sample (before filtration), and in the fluid containing the eluted prions, but not in the filtrate, demonstrating that the prions are present in the spiked fluid, and prions are captured by the filter and eluted from the filter. Additionally, the size and brightness of the bands containing eluted prions is similar to the size and brightness of the bands containing the spiked control, demonstrating the efficiency of capture and release of prions compared to a non-filtered sample.

[0164] The example also shows that at some concentrations (prion dilutions of 1/50, and 1/5), prions can be enriched without the use of PTA.

EXAMPLE 4

[0165] This example demonstrates pathogenic prions can be captured by, and released from, an embodiment of a prion capture/release device according to an embodiment of the invention, wherein the captured pathogenic prions are eluted from the filter using agitation.

[0166] A prion capture/release device is prepared as described in Example 3.

[0167] 10 ml of sheep citric plasma is spiked with 100 μl of 10% SIHBH.

[0168] The 10 ml spiked plasma (pH 7 to 7.6; 22 ± 2°C) is filtered through the prion capture/release device at a flow rate of 1 ml/min. The filtrate is collected. The filter is washed with 5 ml of PBS that is passed through the device at a flow rate of 1 ml/min, and the wash buffer is collected in two aliquots of 2.5 ml each.

[0169] To elute the captured prions, the housing is separated, and the filter is placed in a 10 ml u-bottom tube containing 2 ml of 0.1% sarkosyl in PBS. The tube is vortexed for 10 seconds. 0.5 ml of the obtained material is mixed with 1.0 ml of 4% sarkosyl in PBS. The mixture is then incubated at 37°C for 10 min. Following incubation, 122 μl of 4% PTA

(Sigma PTA Sodium Salt, P-6395) in 170 mM $MgCl_2$ at pH 7.4 is added to the mixture. The resulting mixture is incubated with continuous mixing at 37°C for 30 minutes. The mixture is centrifuged at 18,000 g for 60 minutes at room temperature. After centrifugation the supernatant is discarded and the pellet obtained is resuspended in 500 μl of 0.1% sarkosyl in PBS. The sample is centrifuged a second time at 18,000 g for 60 minutes at room temperature, and the supernatant is removed. The pellet obtained is resuspended in 40 μl of 0.1% sarkosyl in PBS with sonicating for 20-25 sec. After the pellet is resuspended, 10 μl of 250 μg/ml pK is added to the 40 μl sample. The 50 μl sample (containing eluted prions) is then incubated at 52°C for 30 minutes.

[0170] A Western blot is performed as generally described in Example 3.

[0171] The resulting Western blot shows the presence of prions in the spiked plasma sample (before filtration), and in the fluid containing the eluted prions, but not in the filtrate, demonstrating that the prions are present in the spiked fluid, and prions are captured by the filter and eluted from the filter. The blot also shows the presence of prions in the first aliquot of wash buffer, but not the second. It is believed the presence of prions in the first aliquot of wash buffer reflects the presence of residual spiked plasma that was previously held up in the filter.

EXAMPLE 5

[0172] This example demonstrates pathogenic prions that have been enriched using a prion capture/release device are concentrated in accordance with an embodiment of the invention.

[0173] Prions are eluted and processed (mixed with PTA, centrifuged, resuspended, mixed with pK, and incubated) as described in Example 3. Additionally, spiked plasma samples with a similar amount of SIHBH prion sarkosyl/PBS are processed (mixed with PTA, centrifuged, resuspended, mixed with pK, and incubated) as described in Example 3.

[0174] 200 μl samples of eluted prions, and 200 μl spiked plasma samples, are loaded on a NANOSEP® Centrifugal Device (including a 30,000 mwco OMEGA™ polyethersufonce ultrafiltration membrane; Pall Corporation, East Hills, NY). The samples are spun down for 20 minutes at 12,000 g. Following the centrifugation, the samples have a residual volume of 30 μl (the retentate) and a downstream volume of 170 μl (the filtrate). A Western blot is performed.

[0175] The resulting Western blot shows the presence of prions in the retentates, but not in the filtrates, demonstrating that the prions can be concentrated using a spin device in accordance with an embodiment of the invention.

EXAMPLE 6

[0176] This example demonstrates pathogenic prions are captured by, and released from, a prion capture device (wherein non-pathogenic prions are not captured by the device), and are subsequently concentrated in accordance with an embodiment of the invention.

[0177] 96 well ACROPREP™ multiwell devices (Pall Corporation) are obtained. In one device, each well has sealed therein a positively charged membrane having a pore size of about 5 microns. In the other device, each well has sealed therein a positively charged filter (1 layer of fibrous medium) prepared as described in Example 2.

[0178] Prion spiked samples are prepared as described in Example 3.

[0179] Using vacuum suction, a 1 ml sample (pH 7 to 7.6; 22 ± 2°C) of the spiked plasma is filtered through each prion capture/release device at a flow rate of 1 ml/min. The filtrates are collected. Each well is washed with 5 ml of PBS, and the wash is discarded.

[0180] Subsequently, to elute the prions, 100 μL of 0.1% sarkosyl in PBS is pipetted into, and aspirated, from each well, 5 times.

[0181] The obtained eluted material is mixed with 1.0 ml of 4% sarkosyl in PBS. The mixture is then incubated at 37°C for 10 min.

[0182] Following incubation, 122 μl of 4% PTA (Sigma PTA Sodium Salt, P-6395) in 170 mM $MgCl_2$ at pH 7.4 is added to the mixture. The resulting mixture is incubated with continuous mixing at 37°C for 30 minutes. The mixture is centrifuged at 18,000 g for 60 minutes at room temperature.

[0183] After centrifugation the supernatant is discarded and the pellet obtained is resuspended in 500 μl of 0.1% sarkosyl in PBS. The sample is centrifuged a second time at 18,000 g for 60 minutes at room temperature, and the supernatant is removed. The pellet obtained is resuspended in 40 μl of 0.1% sarkosyl in PBS with sonicating for 20-25 sec/sample. After the pellet is resuspended, 10 μl of 250 μg/ml pK is added to the 40 μl sample. This 50 μl sample is then incubated at 52°C for 30 minutes.

[0184] A Western blot is performed.

[0185] The resulting Western blot shows the presence of prions in the spiked plasma sample (before filtration), and in the fluid containing the eluted prions, but not in the filtrate, demonstrating that the prions are present in the spiked fluid, and prions are captured in each well by the membrane filter and the fibrous filter and eluted from the filters.

EXAMPLE 7

[0186] This example demonstrates pathogenic prions that have been enriched using a prion capture/release device are concentrated in accordance with an embodiment of the invention.

[0187] Prions are eluted and processed as described in Example 6.

[0188] 96 well ACROPREP™ multiwell devices (Pall Corporation) are obtained. Each well has sealed therein a 30,000 mwco OMEGA™ polyethersulfone ultrafiltration membrane (Pall Corporation).

[0189] 200 μl samples of eluted prions are loaded on wells. The samples are spun down for 5 minutes at 8,000 g. Following the centrifugation, the samples have a residual volume of 30 μl (the retentate) and a downstream volume of 170 μl (the filtrate). A Western blot is performed.

[0190] The resulting Western blot shows the presence of prions in the retentates, but not in the filtrates, demonstrating that the prions can be concentrated using a multiple well spin device in accordance with an embodiment of the invention.

[0191] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0192] The use of the terms "a" and "an" and "the" and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The terms "comprising," "having," "including," and "containing" are to be construed as open-ended terms (i.e., meaning "including, but not limited to,") unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value

falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0193] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIM(S):

1. A method for concentrating pathogenic prions comprising:

contacting at least one porous medium with a pathogenic prion-containing fluid such that pathogenic prions are retained and/or captured by the porous medium;

contacting the porous medium with another fluid such that pathogenic prions are released from the porous medium to provide a first released pathogenic prion-containing fluid;

placing a fluid containing the released pathogenic prions in a first spin device including a separation medium; and,

centrifuging the spin device to provide a first concentrated pathogenic prion-containing retentate.

2. A system for concentrating pathogenic prions comprising:

a prion capture/release device comprising a housing having at least one inlet and at least one outlet and defining a fluid flow path between the inlet and the outlet; and at least one porous medium across the fluid flow path, the porous medium comprising a pathogenic prions retaining and/or capturing medium; and,

a spin device including a separation medium, wherein the spin device is adapted to be centrifuged to provide a concentrated pathogenic prion-containing retentate.

3. A system for concentrating pathogenic prions comprising:

a prion capture/release device comprising a housing having a plurality of wells, wherein at least two wells each have at least one porous medium sealed therein, the porous medium comprising a pathogenic prions retaining and/or capturing medium; and,

a spin device including a separation medium, wherein the spin device is adapted to be centrifuged to provide a concentrated pathogenic prion-containing retentate.

4. The method of claim 1, wherein contacting the porous medium with the pathogenic prion-containing fluid comprises passing a pathogenic and non-pathogenic

prion-containing fluid through the medium, and wherein pathogenic prions are retained and/or captured by the medium and non-pathogenic prions are essentially not retained and/or captured by the medium.

5. The method of claim 1 or 4, wherein placing the fluid containing the released pathogenic prions in a spin device including a separation medium comprises placing the first released pathogenic prion-containing fluid in the spin device.

6. The method of claim 5, wherein placing the fluid containing the released pathogenic prions in a spin device comprises further processing the first released pathogenic prion-containing fluid to provide the fluid containing the released pathogenic prion placed in the spin device.

7. The method of any one of claims 1 and 4-6, wherein contacting the porous medium with another fluid such that prions are released from the porous medium comprises backflushing the medium with the fluid.

8. The method of any one of claims 1 and 4-7, wherein the separation medium comprises an ultrafiltration membrane.

9. The method of claim 8, wherein the separation medium comprises a 30,000 mwco membrane.

10. The method of claim 8, wherein the separation medium comprises a 100,000 mwco membrane.

11. The method of any one of claims 1 and 4-10, further comprising processing the first concentrated pathogenic prion-containing retentate to provide a second concentrated pathogenic prion-containing retentate.

12. The method of any one of claims 1 and 4-11, wherein the spin device including the separation medium comprises a multiple well device wherein at least two wells each have a separation medium sealed therein.

13. The method of any one of claims 1 and 4-12, wherein contacting the porous medium with another fluid to provide a first released pathogenic prion-containing fluid comprises contacting the medium with a pathogenic conformer releasing solution comprising a detergent.

14. The method of claim 13, wherein the pathogenic conformer releasing solution comprises sarkosyl and at least a 3% salt solution.

15. The method of any one of claims 1 and 4-14, wherein contacting the porous medium with another fluid to provide a first released pathogenic prion-containing fluid comprises backflushing the medium with a pathogenic conformer releasing solution at a flow rate of at least about 2 ml/min.

16. The system of claim 2 or 3, wherein the porous medium captures and/or retains pathogenic prions, and non-pathogenic prions are essentially not retained and/or captured by the medium.

17. The system of any one of claims 2, 3, or 16, wherein the separation medium comprises an ultrafiltration membrane.

18. The system of claim 17, wherein the separation medium comprises a 30,000 mwco membrane.

19. The system of claim 17, wherein the separation medium comprises a 100,000 mwco membrane.

20. The system of any one of claims 2, 3, or 16-19, wherein the spin device including the separation medium comprises a multiple well device wherein at least two wells each have a separation medium sealed therein.

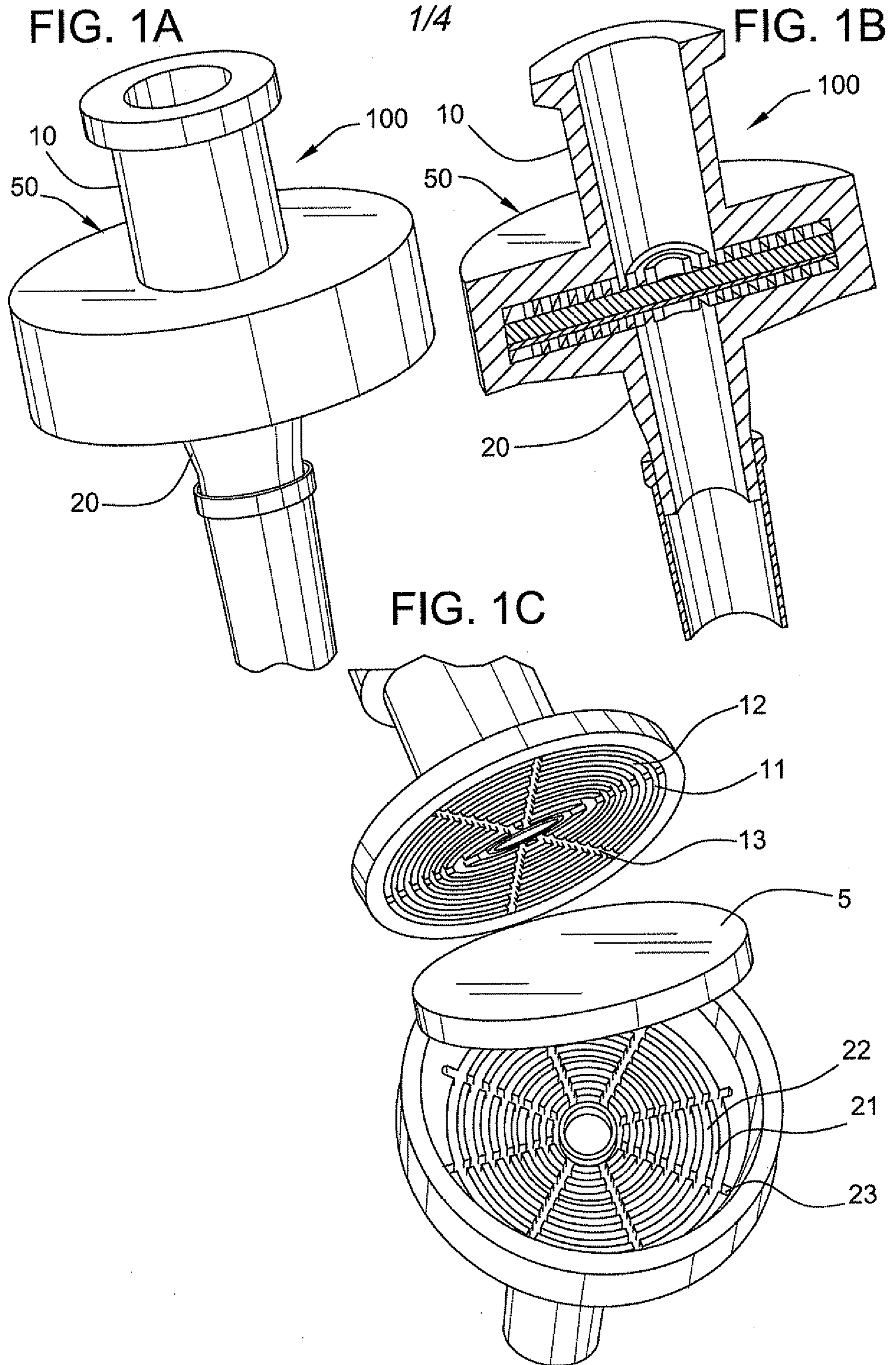
FIG. 1A
10
100
50
20
FIG. 1B
10
100
50
20
FIG. 1C
12
11
13
5
22
21
23

FIG. 2A

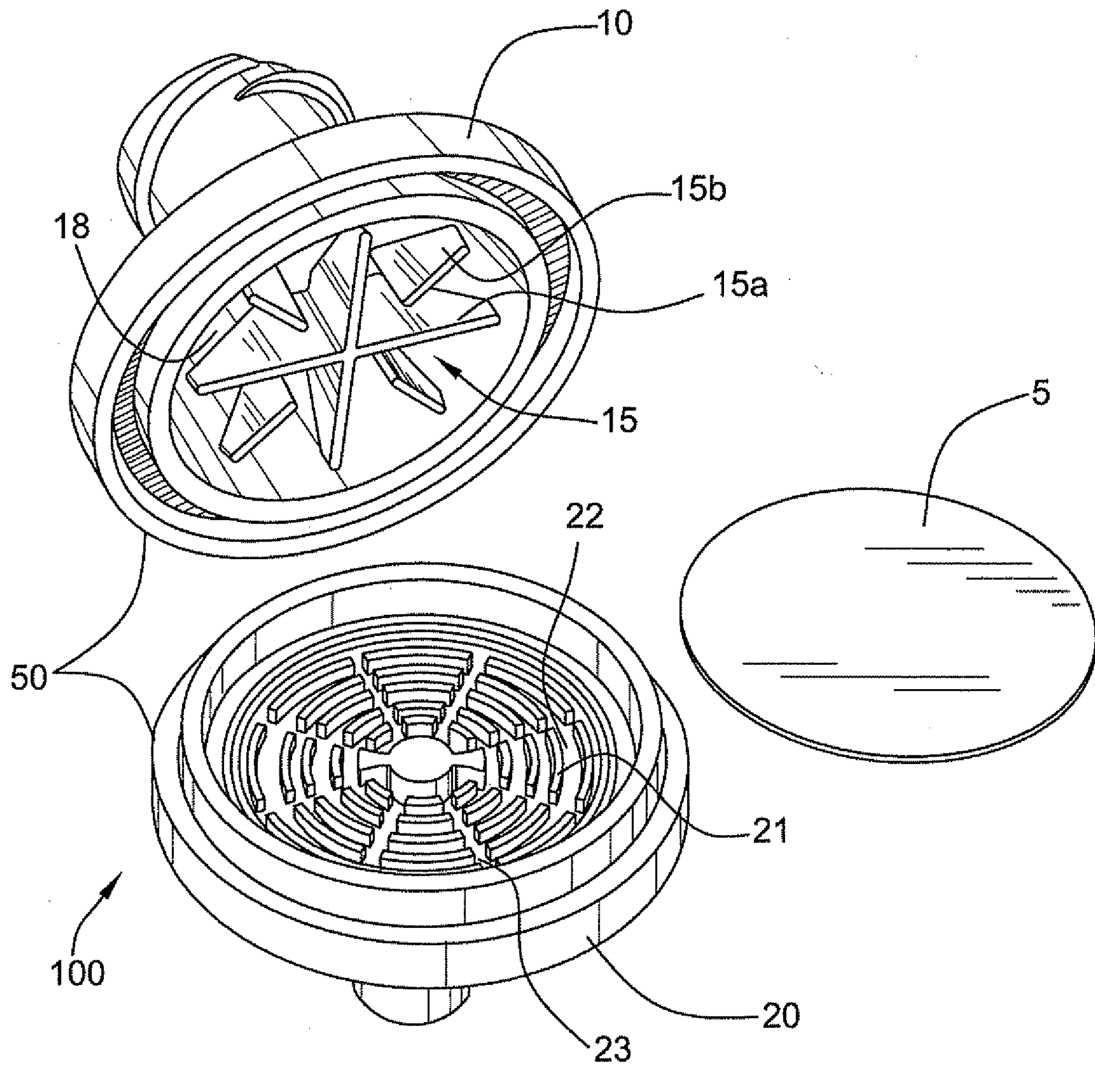

FIG. 2B

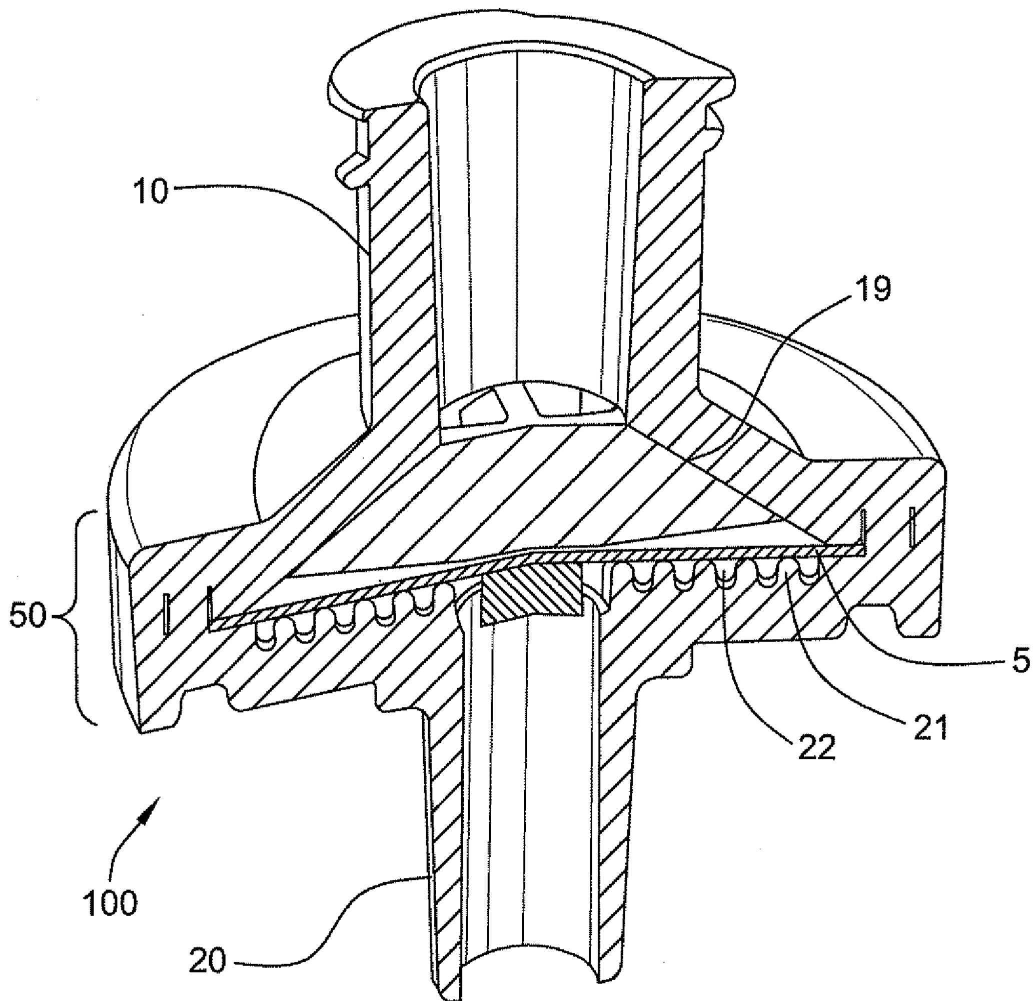

FIG. 3